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(57) Abstract

The inventions provides compositions and a novel method of immunization directed against released transactivating proteins of certain target viruses that are taken up by other cells, including particularly HIV and other integrating and chronically infecting viruses. The method employs *TAT* immunogens, which are capable of eliciting high titer antibody to the native *TAT* protein, especially the regions involved in cellular uptake.

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COMPOSITIONS OF TRANSACTIVATING PROTEINS OF HUMAN IMMUNODEFICIENCY VIRUS

Field of the Invention

The present invention relates generally to methods for immunizing humans or other mammals against infection by viruses that produce proteins that are released extracellularly and are taken up by other noninfected cells, preparing them for infection by the virus.

Background of the Invention

Certain viruses possess genes which are involved in the expression and secretion of viral proteins which function to transfer susceptibility to infection by the virus to other non-infected host cells. One primary example of such a viral gene is the transactivator gene (*tat*) of the human immunodeficiency virus (HIV), which is essential for viral replication. Similar transactivating genes are found in other viruses such as human T cell lymphocytotropic virus (HTLV) I and II, simian immunodeficiency virus (SIV), and feline immunodeficiency virus (FIV).

The *tat* gene of HIV is essential for viral replication; the protein it encodes (TAT) activates transcription of HIV through the HIV long terminal repeat (LTR). The *TAT* protein also activates other host cellular genes which contribute to cellular activation and sustenance of the virus. For example, there is evidence that the *TAT* protein activates host cell genes, such as bcl-2, c-myc, IL-6, TGF-ß, and TNF [G. Zauli et al, Cancer Res., 53:4481-4485 (1993); J. Laurence et al, Proc. Natl. Acad. Sci., USA, 88:7635-7639 (1991); G. Scala et al, J. Exp. Med., 179:961-971 (1994); G. Zauli et al, Blood, 80:3036-3043 (1992); and L. Buonaguro et al, J. Virol., 66:7159-7167 (1992)].

TAT of HIV is also released extracellularly by infected cells and taken up by other non-infected cells [see, e.g., A. D. Frankel et al, Cell, 55:1189-1193 (1988); G. Barillari et al, J. Immunol., 149:3727-3734 (1992); B. Ensoli et al, Nature, 345:84-86 (1990)]. Picomolar to nanomolar extracellular concentrations can be demonstrated to affect the functions of certain cells. Furthermore, *TAT* protein has been shown to selectively depress antigen induced T cell proliferation *in vitro* [R. P. Viscidi et al, Science, 246:1606-1608 (1989)]. This latter immunological abnormality has been demonstrated in T cells from asymptomatic HIV infected subjects early in the disease.

Uptake of *TAT* by cells is very strong, and appears to be mediated by a short basic sequence of the protein. S. Fawell et al, Proc. Natl. Acad. Sci., USA, 91:664-668 (1994) identified this sequence as Tat $_{37-72}$ (CFITKALGISYGRKKRRQRRRPPQGSGTHQVSLSKQ) [SEQ ID NO: 15] and utilized it in fusion proteins to enable the uptake of other proteins into the cells and, more specifically, into the nucleus. If taken up by cells latently infected with HIV, *TAT* protein would likely activate HIV production. If taken up by uninfected cells, *TAT* would likely activate host genes that would render the cell more receptive to fresh HIV infection, thus enabling the expansion of HIV infection in the host.

The *tat* gene and its protein have been sequenced and examined for involvement in proposed treatments of HIV. U. S. Patent No. 5,158,877 discloses synthetic DNA coding for the *TAT* protein of HIV-1, and provides its cDNA sequence. U. S. Patent No. 5,238,882 refers to a transformed yeast cell capable of expressing *TAT* for use in screening for agents which inhibit the function of the protein. U. S. Patent No. 5,110,802 refers to an antiviral agent capable of attacking the first splice

acceptor site of *tat* gene. International Patent Application No. WO92/07871, published May 14, 1992 refers to oligopeptide inhibitors of HIV replication, which operate by direct competition to prevent activation by the *TAT* protein, while International Patent Application No. WO91/10453, published July 25, 1991, refers to inhibition of *TAT* function by DNA sequences. See, also International Patent Application No. WO91/09958, published July 11, 1991.

International Patent Application No. WO87/02989, published May 21, 1987, provides an *E. coli* expression vector for producing *TAT* protein. International Patent Application No. WO92/14755, published September 3, 1992, refers to *in vitro* blocking of *TAT* uptake by a selected integrin cell surface receptor by fragments of *TAT*, fragments of integrin or antibodies to integrin. Also described in this publication is the immunization of rabbits with a *TAT* peptide and Complete Freund's adjuvant to generate antibodies to *TAT* for experimental work. Despite the growing knowledge about the expression and proposed use of *TAT* in HIV therapies, there remains a need in the art for the development of a successful therapeutic or immunization regimen useful in humans (and where appropriate, other mammals) that utilizes active immunization with *TAT* protein or peptides in the treatment or prophylaxis of HIV, as well as for similar regimens utilizing other analogous essential extracellular proteins of other viruses, or their immunogenic peptides.

Summary of the Invention

In one aspect, the invention provides an immunogen capable of eliciting a humoral and/or antibody response in a mammal to which the immunogen is administered, the immune response directed against a native extracellular

transactivating protein from a selected chronic virus, such as HIV. In one embodiment, the immunogen comprises an HIV *TAT* protein sequence or peptide fragment thereof including the sequence involved in cellular uptake of the *TAT*. Hereafter this latter sequence will be referred to as the "cellular uptake region" [in HIV *TAT*, this region spans about AA46 to about AA63 of SEQ ID NO: 2] as defined by S. Pawell et al, cited above. The immunogen itself will be referred to as the *TAT* immunogen. In other embodiments, the immunogen comprises analogous sequences of similar extracellular proteins for other viruses, e.g., SIV, HTLV, and the like. Preferably, this immunogenic protein or peptide sequence has the characteristic of inactivated biological function vis-a-vis the intact *TAT* protein.

In another aspect, the invention provides a DNA sequence encoding the *TAT* immunogen described above for use as a 'naked DNA' composition capable of eliciting an immune response in an animal, preferably a mammal, to which it is administered.

In still a further aspect, the invention provides a DNA molecule containing regulatory sequences which control the replication and expression of the inserted DNA sequence encoding the HIV *TAT* immunogen. Such regulatory sequences may direct the expression of the *TAT* immunogen in cell cultures for recombinant production and manipulation of the DNA. Alternatively, such a vector may also be administered as an immunogenic composition, such as a vaccine, for expression of the *TAT* immunogen *in vivo* in a host mammal and the elicitation of an immune response thereto.

In still a further aspect, the invention provides immunogenic compositions, such as vaccine compositions, useful for producing an immune response to, and for immunizing a patient against infection with, a virus

characterized by a transactivating protein or analogous extracellular protein. One embodiment of such an immunogenic composition comprises the protein or peptide immunogen described herein in a suitable pharmaceutical carrier. Another embodiment of the immunogenic composition comprises a DNA sequence capable of expressing the immunogen *in vivo* upon direct administration of the DNA into a host animal. In still another embodiment of such an immunogenic composition, the DNA sequence encoding the immunogen is present in a vector with associated regulatory sequences.

Still a further aspect of this invention is a method for producing an immune response, such as a protective immune response, in a patient against infection with a virus characterized by a transactivating or analogous extracellular protein. The method comprises administering to said patient an HIV *TAT* immunogen or *tat* nucleic acid sequence as described above in an amount sufficient to evoke production of high titer antibodies capable of specifically binding the native HIV *TAT* protein secreted by said virus. Preferably the antibodies would bind to the cellular uptake region of the *TAT* protein.

In yet a further aspect, the invention contemplates the production of analogous methods and compositions designed similarly for other proteins which function in a manner similar to that of the *TAT* protein of HIV, including proteins of other viruses. Thus this method and compositions are anticipated to be useful in the prophylaxis of various chronic viruses including, HIV-1, HTLV-I, HTLV-II, HIV-2, SIV, and FIV.

Other aspects and advantages of the present invention are described further in the following detailed description of the preferred embodiments thereof.

Brief Description of the Drawings

Fig. 1 illustrates the cDNA sequence [SEQ ID NO: 1] and amino acid sequence [SEQ ID NO: 2] of the HIV-1 *TAT* protein.

Fig. 2 is a graph illustrating the purification of the I^{125} labelled SIV-Tyr_0 TAT_{85-90} sequence using reverse phase HPLC. The purified label will be used to detect the presence of antibodies to *TAT* following immunization. See, Example 2 below.

Detailed Description of the Invention

The present invention provides novel immunogens, immunogenic compositions, vaccine compositions, and methods of eliciting an immune response in mammals, particularly humans, against viruses that produce proteins that are released extracellularly, which proteins are in turn taken up by other uninfected, or latently infected, cells, thereby rendering the uninfected cells susceptible to viral infection. One such viral protein capable of facilitating infectivity from one cell to another uninfected cell is the transactivating *TAT* protein of HIV (and in the monkey virus, SIV); other such proteins exist in other viruses.

For ease of discussion, the following description relates to the *TAT* proteins of HIV and SIV as specific examples of extracellular proteins which perform the above-described role of perpetuating viral infection. However, it should be understood by one of skill in the art that analogous extracellular proteins necessary for the development and spread of the viral infection in HIV, SIV, and other viruses may be manipulated similarly to provide other immunogenic compositions and methods encompassed by this invention.

While not wishing to be bound by theory, the inventors have determined that the novel method of this

invention and the related compositions herein described permit the elicitation of an immune response in a mammal directed against these extracellular or transactivating proteins. Because infection is perpetuated either by activation of latently infected cells or by activation of host genes of uninfected cells which render the cells susceptible to viral infection, the mechanism underlying this invention is significant.

An important feature of the invention and an advantage over other therapeutic or vaccinal compositions useful for the treatment of viral infections, such as HIV, is that this invention elicits and directs an immunized host's immune response against the extracellular *TAT* protein. Active immunization against a selected virus, e.g., HIV-1, may be accomplished by eliciting high titer antibodies that will complex with the native secreted *TAT* protein produced by the virus and prevent its uptake by cells. That is, once *TAT* protein has been expressed by the virus in an infected cell and is separated from the virus itself and is secreted from the infected cell in which it was produced, the immune response produced by the methods and compositions of this invention interdicts the *TAT* protein extracellularly before it can be taken up by another cell.

This immunization has a particularly desirable advantage in contrast to other treatments and prophylactic methods employed against such viruses. Because the immunization is not directed against the virus itself, interdiction of the *TAT* protein extracellularly does not create a selective pressure on the parent virus itself, which would encourage the development of mutant virus variants producing a *TAT* protein that is not recognized by the induced antibodies.

However, any viral strain which is not producing the same *TAT* protein, or an immunologically cross-reactive

protein, will be unaffected by the use of the immunogenic compositions of this invention. In this case treatment with another immunogenic composition reactive with that *TAT* protein and prepared according to this invention is contemplated. Additionally, it is anticipated that blocking the incorporation of *TAT* protein by the patient's cells may also reduce the level of viremia.

A. Protein/Peptide Immunogens of the Invention

One embodiment of this invention involves the development of a selected *TAT* immunogen. By "immunogen" is meant any molecule which elicits an immune response, either cellular or humoral, in an animal exposed to that molecule *in vivo*. An immunogen of this invention is desirably an HIV *TAT* protein or peptide fragment thereof, which comprises cellular uptake region of the *TAT* protein or fragments thereof. The cellular uptake region is that region which mediates the binding of *TAT* protein to the cell to be infected. One variant of the HIV *TAT* protein is illustrated in SEQ ID NO: 2 and in Fig. 1; the cellular uptake region is identified as spanning amino acids 46 to 63 of that sequence SYGRKKRRZRRRAPZGSQ of SEQ ID NO: 2. Preferably, the cellular uptake region includes a smaller fragment, such as amino acids 46 through 59 thereof. However it is known that other variants of HIV exist which differ from that sequence at one or more amino acids. Thus, the cellular uptake region as defined herein can include all or a portion of the sequence identified above, as well as variants thereof.

Preferably, the *TAT* protein or peptide fragment useful as the immunogen is biologically inactive; i.e., it does not share the biological function of the intact *TAT* protein. The immunogen may be made inactive by deletion of amino acids at the amino terminus or carboxy

terminus, or the deletion or replacement of native Cysteine residues to interfere with naturally-occurring disulfide bonds in the protein. One embodiment of a *TAT* immunogen may consist of a *TAT* protein characterized by deletion of at least 3 or more amino acids at the amino terminus.

Additional embodiments of a *TAT* protein immunogen include a peptide fragment from a selected region of *TAT* protein, which peptide fragment alone is biologically inactive, and may be optionally coupled to a carrier. A desired size for such an immunogenic peptide may be between about 12 to about 22 amino acids. However, other sizes may be desired, depending on the peptide construct, i.e., for a multiple antigenic peptide (also referred to as an octameric lysine core peptide) as described in detail below, the peptide may desirably be about 20 amino acids in length.

A selected region of the *TAT* immunogen of this invention may be a conserved region or an immunogenic region of *TAT* protein. Preferably, this peptide sequence includes the cellular uptake region. For example, where the virus selected for use in this invention is HIV-1, the selected *TAT* immunogen may include, among other sequences, the following peptide sequences and variants:

SYGRKKRRQRRRAPQGSQ [SEQ ID NO: 3]
SYGRKKRRQRRRAP [SEQ ID NO: 4]
SYGKKKRRQRRRAP [SEQ ID NO: 5]
SYGRKKRKQRRRAP [SEQ ID NO: 6]
SYGRKKRRPRRRAP [SEQ ID NO: 7]
SYGRKKRRQRQRAP [SEQ ID NO: 8]
SYGRKKRRQRRGAP [SEQ ID NO: 9]
SYGRKKRRQRRRTP [SEQ ID NO: 10]
SYGRKKRRQRRRPP [SEQ ID NO: 11]
SYGRKKRRQRRRAH. [SEQ ID NO: 12]

Larger peptides incorporating the above-identified sequences of amino acids of *TAT* protein of HIV or smaller fragments and fragments thereof may also be employed to form the *TAT* immunogen of this invention. For example, smaller fragments of the cellular uptake region, such as QRRRAP [SEQ ID NO: 16] and its variants or GRKKRRQ [SEQ ID NO: 17] and its variants may also be employed alone or repeated in one peptide in multiple antigenic peptides. Similarly, where the virus is other than HIV-1, e.g., a non-human virus, such as SIV, one of skill in the art is expected to be able to select analogous regions of the transactivating or analogous extracellular protein of the virus for use as an immunogen in accordance with this invention. For example, where the virus selected for use in this invention is SIV, the selected *TAT* protein region may include, among other sequences, the cellular uptake region occurring at about amino acids 79 through about amino acids 100 of the SIV *TAT* protein, as well as the following peptide sequences and variants:

RRRTPKKTKANTSSASY [SEQ ID NO:13] which spans the SIV *TAT* sequence from amino acids 80 through 95; and

YEQQRRRTPKKTKANTSSAS [SEQ ID NO:14], which spans SIV TAT_{76-95}. Other variants in size and amino acid composition may also be employed in this invention, such as the peptide motifs PKKTK [SEQ ID NO: 18], PKKAK [SEQ ID NO: 19] and QRRRTP [SEQ ID NO: 20] alone or repeated in a single peptide, desirably in multiple antigenic peptides as disclosed below.

In a preferred embodiment, the immunogen of the invention is in the form of a multiple antigenic peptide construct comprising a plurality of peptides from a *TAT* protein. For example, such a construct may be designed employing the multiple antigenic peptide system described by Tam, Proc. Natl. Acad. Sci. USA, 85:5409-5413 (1988). This system makes use of a core matrix of lysine residues

onto which multiple copies of an immunogenic peptide are synthesized [D. Posnett et al, J. Biol. Chem., 263(4):1719-1725 (1988); J. Tam, "Chemically DefinedSynthetic Immunogens and Vaccines by the Multiple Antigen Peptide Approach", Vaccine Research and Developments, Vol. 1, ed. W. Koff and H. Six, pp. 51-87 (Marcel Deblau, Inc., New York 1992)]. These constructs are designed for use in the present invention, as described in detail in Example 1 below. Other conventional protein/peptide immunogenic constructs may be designed by resort to known techniques.

In addition to the amino acid sequences of the specifically-recited HIV-1 *TAT* proteins described herein, the invention also encompasses immunogens prepared from other amino acid sequences of viral transactivating or analogous extracellular proteins. For example, selected *TAT* peptides may be developed from allelic or engineered variations of viral genes or gene fragments encoding the particular *TAT* protein. The immunogens employed in this invention may also have amino acid sequences which are analogs or derivatives of *TAT* or related extracellular protein sequences of a selected virus.

The immunogens of this invention may also be prepared to reflect variations in the native DNA and amino acid sequences of selected *TAT* proteins. For example, such immunogens may typically include *TAT* protein analogs, and the DNA encoding them, that differ by only 1 to about 4 codon changes. Other examples of analogs include polypeptides with minor amino acid variations from the natural amino acid sequence of *TAT* proteins, in particular, conservative amino acid replacements.

Conservative replacements are those that take place within a family of amino acids that are related in their side chains. Genetically encoded amino acids are

generally divided into four families: (1) acidic = aspartic acid, glutamic acid; (2) basic = lysine, arginine, histidine; (3) non-polar = alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan; and (4) uncharged polar = glycine, asparagine, glutamine, cysteine, serine, threonine, tyrosine. Phenylalanine, tryptophan, and tyrosine are sometimes classified jointly as aromatic amino acids. For example, it is reasonable to expect that an isolated replacement of a leucine with an isoleucine or valine, an aspartate with a glutamatic acid, a threonine with a serine, or a similar conservative replacement of an amino acid with a structurally related amino acid will not have a significant effect on peptide structure or activity.

Preferably, the *TAT* protein/peptide immunogens of this invention are prepared by chemical synthesis techniques, such as described by Merrifield, J. Amer. Chem. Soc., 85:2149-2154 (1963), and including the multiple antigenic techniques described above. Alternatively, they may be prepared by known recombinant DNA techniques by cloning and expressing within a host microorganism or cell a DNA fragment carrying a coding sequence for the selected *TAT* immunogen. Coding sequences for the *TAT* protein fragments can be prepared synthetically or can be derived from viral RNA by known techniques, or from available cDNA-containing plasmids.

Systems for cloning and expressing the *TAT* immunogen in various microorganisms and cells, including, for example, E. coli, Bacillus, Streptomyces, Saccharomyces, mammalian, yeast and insect cells, and suitable vectors therefor are known and available from private and public laboratories and depositories and from commercial vendors. Currently, the most preferred host is a mammalian cell such as Chinese Hamster ovary cells (CHO) or COS-1 cells. These hosts may be used in connection

with poxvirus vectors, such as vaccinia or swinepox. The selection of other suitable host cells and methods for transformation, culture, amplification, screening and product production and purification can be performed by one of skill in the art by reference to known techniques. See, e.g., Gething and Sambrook, Nature, 293:620-625 (1981).

Another preferred system includes the baculovirus expression system and vectors. Bacterial expression may also be desired.

When produced by conventional recombinant means, the *TAT* immunogens may be isolated either from the cellular contents by conventional lysis techniques or from cell medium by conventional methods, such as chromatography. See, e.g., Sambrook et al, Molecular Cloning. A Laboratory Manual., 2d Edit., Cold Spring Harbor Laboratory, New York (1989).

The resulting *TAT* protein or peptide immunogens, or multiple antigenic peptides may be screened for efficacy as a immunogen by *in vivo* assays, employing immunization of an animal, e.g., a simian, with the *TAT* protein or fragment, and evaluation of titers of antibody to the native *TAT* protein of the selected virus. Examples 3 and 4 disclose suitable assay systems for such evaluation.

B. 'Naked DNA' Compositions of the Invention

Still another aspect of this invention involves nucleic acid sequences which encodes the *TAT* protein immunogens described above. The nucleic acid sequences, such as that described as SEQ ID NO: 1 or allelic variants thereof, or DNA sequences containing certain preference codons for the species of the indicated patient preferably in the form of DNA, may be used as so-called 'naked DNA' to express the protein/peptide immunogen *in vivo* in a patient. See, e.g., J. Cohen,

Science, 259:1691-1692 (March 19, 1993); E. Fynan et al, Proc. Natl. Acad. Sci., USA, 90:11478-11482 (Dec. 1993);and J. A. Wolff et al, Biotechniques, 11:474-485 (1991), all incorporated by reference herein.

The DNA sequences encoding the selected *TAT* immunogen of this invention may also be employed in a plasmid for either direct injection into a host animal or in a vector under suitable regulatory sequences for expression of the protein *in vitro*. The latter form of expression is conventional (see, Sambrook et al, cited above and the references above to production of the protein). The former type of plasmid for *in vivo* expression of the immunogen may be designed as described in the references above or, e.g., as described in International Patent Application PCT WO94/01139, published January 20, 1994. Briefly, the DNA encoding the *TAT* protein or desired fragment thereof is inserted into a nucleic acid cassette. This cassette is engineered to contain, in addition to the *tat* sequence to be expressed, other optional flanking sequences which enable its insertion into a vector. This cassette is then inserted into an appropriate DNA vector downstream of a promoter, an mRNA leader sequence, an initiation site and other regulatory sequences capable of directing the replication and expression of that sequence *in vivo*. This vector permits infection of patient's cells and expression of the *TAT* immunogen *in vivo*.

C. Immunogenic Compositions of the Invention

Thus, the present invention provides immunogenic compositions containing a *TAT* protein or peptide immunogen of the invention or the 'naked DNA' of the invention. These immunogenic compositions are capable of eliciting in an immunized host mammal, e.g., a human, an

immune response capable of interdicting extracellular *TAT* protein.

In one embodiment, such immunogenic compositions contain at least one such protein/peptide immunogen directed to a selected native *TAT* protein according to the invention or a fragment thereof, together with a carrier suitable for administration as a composition for prophylactic treatment of virus infections. Alternatively, the compositions may contain more than one immunogen, the amino acid sequences of the immunogens differing to encompass allelic variants of the *TAT* protein. For example, a multiple antigenic peptide of the invention may contain repeats of the same peptide, e.g., for HIV, RRRAP [SEQ ID NO: 21]. The composition may preferably contain a number of MAPs, each bearing copies of different *TAT* peptides as described herein.

Further, where the occurrence of more than one strain of a particular virus is suspected, which strain produces a different *TAT* protein, novel *TAT* immunogens may be designed by resort to this disclosure. For example, the HIV strain of a patient not responsive to a composition of this invention may be isolated and the *TAT* protein sequence determined by conventional methods, e.g., polymerase chain reaction. If the sequence is indeed different from that sequence which was employed in the first composition, another *TAT* immunogen may be obtained and administered with the first immunogen in a single immunogenic composition. Thus, an immunogenic or vaccine composition of this invention may contain several different *TAT* immunogens of this invention, each directed to a different *TAT* protein produced as described herein.

The *TAT* immunogen(s) of the invention or fragments thereof can be employed in a vaccine composition containing a carrier, such as saline, and a selected adjuvant, such as aqueous suspensions of aluminum and

magnesium hydroxides, liposomes and others. Such compositions may optimally contain other conventionalvaccine components. Other suitable carriers and adjuvants are known to the art and the selection thereof is expected to be routine. The preparation of a pharmaceutically acceptable vaccine composition, having appropriate pH isotonicity, stability and other conventional characteristics is within the skill of the art.

These immunogenic or vaccine compositions according to the present invention can be administered by an appropriate route, e.g., by the oral, intranasal, subcutaneous, intravenous, intraperitoneal or intramuscular routes. The presently preferred route of administration is intramuscular.

The amount of the *TAT* immunogen of the invention present in each vaccine dose is selected with regard to consideration of the patient's age, weight, sex, general physical condition and the like. The amount required to induce an immune response, preferably a protective response, in the patient without significant adverse side effects may vary depending upon the immunogen employed and the optional presence of an adjuvant. Generally, it is expected that each dose will comprise between about 50 μg to about 1 mg of *TAT* protein/peptide immunogen per mL of a sterile solution. A more preferred dosage may be about 200 μg of *TAT* immunogen of this invention. Other dosage ranges may also be contemplated by one of skill in the art. Initial doses may be optionally followed by repeated boosts, where desirable.

Alternatively, immunogenic compositions of this invention may be designed for direct administration of 'naked DNA' encoding one or more *TAT* immunogens of this invention. As discussed above with reference to the recited art on the administration of DNA vaccines, it is

anticipated that nucleic acid sequences encoding one or more desired *TAT* immunogens of this invention may be directly delivered into the patient, alone or as part of a plasmid. Suitable vehicles for direct DNA administration include, without limitation, saline, or sucrose, protamine, polybrene, polylysine, polycations, proteins, $CaPO_4$ or spermidine. See e.g, PCT application WO94/01139 and the references cited above. As with the protein immunogenic compositions, the amounts of components in the 'naked DNA' composition and the mode of administration, e.g., injection or intranasal, may be selected and adjusted by one of skill in the art.

Thus, the immunogenic compositions of this invention are designed to prevent infection by the selected virus of an uninfected mammal, e.g., human. Such immunogenic compositions thus have utility as vaccines.

D. Therapeutic Uses of the Invention

It is also further anticipated that the compositions of this invention may also be employed therapeutically to treat already infected patients. The mechanism of the present invention as described above may be useful in impeding the course of viral infection and producing desirable clinical results. More specifically, the compositions of this invention are capable of reducing viremia in patients already infected with the virus by blocking further uptake of the *TAT* protein by uninfected cells. In conjunction with other therapeutic regimens for HIV infected patients, for example, the compositions of the present invention are anticipated to assist in the reduction of viremia and prevention of clinical deterioration. For such therapeutic uses, the formulations and modes of administration are substantially identical to those described specifically above and may be administered concurrently or

simultaneously with other conventional therapeutics for the specific viral infection. For therapeutic use, repeated dosages may be desirable.

E. Other Utilities of the TAT Immunogens of this Invention

As with other peptides, proteins, and nucleic acid sequences in general, the *TAT* immunogens of this invention may have a number of other utilities. For example, the *TAT* peptide or protein immunogens of this invention may be employed in conventional assays, e.g., Western blots, ELISA, and other protein binding assays, for use in identifying antibodies which are capable of detecting HIV strains with different extracellular *TAT* proteins.

Similarly, the DNA sequences encoding the *TAT* immunogens or complementary strands thereto may be used in nucleic acid assays, such as Northern and Southern blots, and other nucleic acid hybridization assays for the same purpose. The nucleic acid sequences encoding the *TAT* immunogens of the invention may be employed as polymerase chain reaction sequences to identify HIV strains on the basis of the sequence of the *tat* gene.

Other uses for the proteins are in the development of antibodies which are directed to the cellular uptake regions.

Polyclonal antibodies may be produced by conventional methods, i.e., by injecting a laboratory animals with sufficient quantities of the *TAT* protein or fragments thereof and collecting the antibodies from the animal's tissue.

A "monoclonal antibody" refers to homogenous populations of immunoglobulins which are capable of specifically binding to the *TAT* protein of this invention. It is understood that the *TAT* protein may

have one or more antigenic determinants. The antibodies of the invention may be directed against one or more of these determinants.

Hybridomas capable of secreting these monoclonal antibodies are produced by conventional techniques by employing as antigen, the *TAT* protein described herein. Generally, the hybridoma process involves generating a B-lymphocyte which produces a desired polyclonal antibody. Techniques for obtaining the appropriate lymphocytes from mammals injected with the target antigen are well known. Generally, the peripheral blood lymphocytes (PBLs) are used if cells of human origin are desired. If non-human sources are desired, spleen cells or lymph nodes from other mammalian sources are used. A host animal, e.g. a rabbit, is injected with repeated doses of the purified antigen, and the mammal is permitted to generate the desired polyclonal antibody producing cells.

Thereafter the B-lymphocytes are harvested for fusion with the immortalizing cell line. Immortalizing cell lines are usually transformed mammalian cells, particularly cells of rodent, bovine and human origin. Most frequently, rat or mouse myeloma cells are employed. Techniques for fusion are also well known in the art and generally involve mixing the cells with a fusing agents, e.g. polyethylene glycol.

Immortalized hybridoma cell lines are selected by standard procedures, such as HAT selection. From among these hybridomas, those secreting the desired monoclonal antibody are selected by assaying the culture medium by standard immunoassays, such as Western blotting, ELISA, or RIA. Antibodies are recovered from the medium using standard purification techniques. See, generally, Kohler et al, Nature, 256:495 (1975) for antibody production techniques. Alternatively, non-fusion techniques for

generating an immortal antibody-producing hybridoma cell line is possible, e.g. virally induced transformation.

Recombinant techniques, such as described by Huse et al, Science, 246:1275-1281 (1988), or any other modifications thereof known to the art may also be employed to produce recombinant antibodies and chimeric antibodies, where desired.

These antibodies are likewise useful in diagnostic methods for detecting and identifying different HIV strains based on the differences in their extracellular *tat* genes. These antibodies may perform such detection in conventional assays known to one of skill in the art.

Other utilities are conventional and may include use of the *TAT* protein and peptide immungens as molecular weight markers.

The following examples illustrate preferred methods for preparing *TAT* immunogens of the invention and utilizing these immunogens to induce immune response to the virus in an immunized host. These examples are illustrative only and do not limit the scope of the invention.

EXAMPLE 1 - SYNTHESIS OF PEPTIDES OF THE INVENTION - SIV TAT AND HIV-1 TAT SEQUENCES

The peptides of the SIV *TAT* and HIV-1 *TAT* were synthesized on an Applied Biosystems Model 430A peptide synthesizer by the solid-phase method developed by Merrifield, cited above. Peptide assembly was carried out starting with the p-methylbenzhydrylamine resin andthe Boc-protected amino acids using a symmetrical anhydride activation procedure. All the amino acids were double coupled to ensure complete coupling. Side chain protecting groups were as follows: benzyl ether (Bzl) for the hydroxyl group of threonine and serine; benzyl ester

for the carboxyl group of aspartic and glutamic acids; tosyl (Tos) for the guanidine of arginine; benzyloxymethyl (Bom) for the imidazole of histidine; 2-chlorobenzyloxycarbonyl (Cl-Z) for the ϵ-amine of lysine; 2-bromobenzyloxycarbonyl (Br-Z) for the phenolic hydroxyl of tyrosine. Asparagine, glutamine, and arginine were coupled as their 1-hydroxybenzotriazole esters.

A. Synthesis of Tyr-Arg-Arg-Arg-Thr-Pro-Lys-Lys-Thr-Lys-Ala-Asn-Thr-Ser-Ser-Ala-Ser-NH_2:[Tyr_0-SIV *TAT*$_{80-95}$]-NH_2 [SEQ ID NO: 22]

The protected peptide, Boc-Tyr(Br-Z)-Arg(Tos)-Arg(Tos)-Arg(Tos)-Thr(Bzl)-Pro-Lys(Cl-Z)-Lys(Cl-Z)-Thr(Bzl)-Lys(Cl-Z)-Ala-Asn-Thr(Bzl)-Ser(Bzl)-Ser(Bzl)-Ala-Ser(Bzl)-(Me-BHA)-resin was synthesized by the symmetrical anhydride coupling technique, utilizing standard coupling protocols (Std 1 cycle, version 1.40) on the ABI 430A peptide synthesizer. [Note that the initial Tyr is referred to as Tyr_0, since the first SIV *tat* amino acid is Arg (SIV *TAT* protein amino acid 80). The Tyr is added to permit ligand attachment.] The synthesis was initiated with p-methylbenzhydrylamine resin (0.6 mmole, 780 mg, 0.77 meq/g). The N-terminal Boc-group was removed by the end-NH_2 program (version 1.40). The resin was then washed with DMF (5 X 10 mL) and CH_2Cl_2 (5 X 10 mL), and dried in a vacuum oven at 30°C (2.96 g).

The peptide was cleaved from the resin support (1.48 g) by stirring in liquid HF (50 mL), p-cresol (1.2 mL), p-thiocresol (1.2 mL), and dimethyl sulfide (1.2 mL) for 1 hour at 0°C. After the removal of excess HF under reduced pressure, the resin-peptide mixture was extracted with anhydrous diethyl ether (3 x 200 mL). The ether extracts were discarded. The cleaved peptide was then extracted with 30% aqueous acetic acid (3 X 70 mL). After removal of solvents under reduced pressure, the

residue obtained was dissolved in water and freeze-dried. The crude peptide was dissolved in 30% acetic acid and passed through an Amberlite IRA-68 (acetate form) ion exchange column (60 g, 1.6 meq/mL, 2.73 cm i.d. X 18 cm length) in 30% aqueous HOAc at a flow rate of 60 mL/h. The appropriate fractions from two such experiments were combined and freeze-dried (1.59 g).

The crude peptide was dissolved in 0.1% TFA/H_2O and purified by preparative RP-HPLC using a Vydac 218TP1022 column (22 X 250 mm). The mobile phases employed were as shown below:

A = 0.1% TFA/H_2O

B = 0.1% TFA/CH_3CN - H_2O, 4:1, v/v

A linear gradient of 0% B to 8% B over 80 minutes at a flow rate of 8 mL/minute was used. The fractions were analyzed by HPLC and those containing pure peptide were combined and the organic solvents were removed under reduced pressure. The residue was dissolved in water, converted into the acetate form by passing through an ion-exchange column, and freeze-dried (327 mg).

Thin layer chromatography (TLC) was performed on Merck F-254 plates (5 X 10 cm) in the following solvent systems (v/v):

Rf(1) = 0.65 (1-BuOH:Pyr:HOAc:H_2O, 5:4:8:2)

Rf(2) = 0.25 (1-BuOH:Pyr:HOAc:H_2O, 5:4:4:2)

Amino acid analysis (AAA):

Ala 2.16 (2), Arg 3.23 (3), Asx 1.23 (1), Lys 3.21 (3), Pro 1.07 (1), Ser 2.27 (3), Thr 2.77 (3), Tyr 1.09 (1). During the hydrolysis of the peptide, Ser undergoes decomposition resulting in low recovery.

Liquid Chromatography - Mass Spectrometry (LC-MS):

$[MH^+]$ at m/z = 651.9 a.m.u., charge 3+; 977.1 a.m.u., charge 2+. Observed mol. wt. 1952 (Theoretical mol. wt. 1951.2).

B. Synthesis of Ser-Tyr-Gly-Arg-Lys-Lys-Arg-Arg-Gln-Arg-Arg-Arg-Ala-Pro-Gln-Gly-Ser-Gln-NH_2: [HIV-1 *TAT*$_{46-63}$]-NH_2 [SEQ ID NO: 3]

This peptide was synthesized as described above, using the appropriate protected amino acid derivatives. The characteristics of the peptide are as follows.

TLC: Rf(1) = 0.24 (1-BuOH:Pyr:HOAc:H_2O, 5:4:8:2, v/v)

Rf(2) - 0.09 (1-BuOH:Pyr:HOAc:H_2O, 5:4:4:2, v/v)

AAA: Ala 1.71 (1), Arg 5.85 (6), Glx 2.97 (3), Gly 1.87 (2), Lys 2.03 (2), Pro 1.37 (1), Ser 1.30 (2), Tyr 0.93 (1).

LC-MS: $[MH^+]$ at m/z = 1108.2 a.m.u., charge 2+. Observed mol. wt. 2214.4 (Theoretical mol. wt. 2214.5).

EXAMPLE 2 - SYNTHESIS OF MULTIPLE ANTIGENIC PEPTIDES OF THE SIV *TAT* AND HIV-1 *TAT* SEQUENCES

Traditionally, small peptides have been conjugated to larger carrier proteins or self-polymerized to render them immunogenic. Both of these techniques suffer from significant limitations that reduce their utility for the production of highly specific antibodies. These obstacles were overcome by using the multiple antigen peptide system of D. N. Posnett et al, J. Biol. Chem., 263:1719-1725 (1988). The peptide was synthesized directly onto a branching lysine core with 8 copies of the peptide sequence linked to the core by the carboxy

terminal amino acid. The octameric multiple antigenic peptide was highly immunogenic in mice and rabbits, allowing production of monoclonal and polyclonal antibodies of high titer. The resultant antibodies were capable of recognizing the specific peptide sequence within a larger synthetic peptide fragment or natural SIV *TAT* protein.

A. Synthesis of Multiple Antigenic Peptide: [(Boc)-Lys(Boc)]$_4$-Lys$_2$-Lys-Gly-OCH$_2$-PAM Resin

The synthesis of a multiple antigenic peptide was accomplished manually by a stepwise solid-phase procedure on Boc-Gly-OCH$_2$-PAM resin. The synthesis was initiated with 1.5 mmoles (1.97 g, 0.76 mmol/g) of the resin. The synthesis of the first and every subsequent level of the carrier core was achieved using a 3 molar excess of Boc-Lys(Boc)-OH. All the couplings were done by the DCC-HOBt method following the protocol given below:

Step	Reagent	Time (minutes)
1.	CH_2Cl_2 wash	3 X 1 min.
2.	40% TFA - 10% anisole in CH_2Cl_2	1 X 5 min.
3.	40% TFA - 10% anisole in CH_2Cl_2	1 X 25 min.
4.	CH_2Cl_2 wash	3 X 1 min.
5.	10% NMM in CH_2Cl_2	2 X 5 min.
6.	CH_2Cl_2 wash	3 X 1 min.
7.	DMF wash	2 X 1 min.
8.	Boc-Lys(Boc)-OH (4.5 mmol) and HOBt (1.5 mmol) in DMF	1 X 3 min.
9.	DIC (4.5 mmol) added to the above and shaken	1 X 180 min.
10.	Recouple, if necessary by repeating steps 4-9	
11.	DMF wash	3 X 1 min.

After three successive couplings of Boc-Lys(Boc)-OH, the resin was washed with DMF (3 X 50 mL) and CH_2Cl_2 (3 X 50 mL) and dried in a vacuum oven at 30°C (3.71 g).

B. Synthesis of [Arg-Arg-Arg-Thr-Pro-Lys-Lys-Thr-Lys-Ala-Asn-Thr-Ser-Ser-Ala-Ser]$_8$-Lys$_4$-Lys$_2$-Lys-Gly-OH: [(SIV *TAT*$_{80-95}$) Multiple Antigenic Peptide] [SEQ ID NO: 23]

The protected precursor of (SIV *TAT*$_{80-95}$) multiple antigenic peptide, [Boc-Arg(Tos)-Arg(Tos)-Arg(Tos)-Thr(Bzl)-Pro-Lys(Cl-Z)-Lys(Cl-Z)-Thr(Bzl)-Lys(Cl-Z)-Ala-Asn-Thr(Bzl)-Ser(Bzl)-Ser(Bzl)-Ala-Ser(Bzl)]$_8$-Lys$_4$-Lys$_2$-Lys-Gly-OCH$_2$-PAM resin was synthesized by growth from both the α and ε amino groups using the symmetrical anhydride coupling technique, utilizing the standard coupling protocols (std 1 cycle, software version 1.40) on the ABI 430A peptide synthesizer. All the amino acids were double coupled to ensure complete coupling. The synthesis was initiated with [Boc-Lys(Boc)]$_4$-Lys$_2$-Lys-Gly-OCH$_2$-PAM resin (0.22 mmol, 94 mg) prepared as described in A. above. The N-terminal Boc group was removed by the end-NH_2 program (version 1.40). The resin was then washed with DMF (5 X 10 mL) and CH_2Cl_2 (5 X 10 mL), and dried in a vacuum oven at 30°C (1.9 g).

The protected peptide-resin was treated with liquid hydrogen fluoride, in the presence of p-cresol, p-thiocresol and dimethylsulfide as scavengers, at 0°C for 1 hour with constant stirring. Excess HF was removed by vacuum and the residue treated with ether to remove scavenger products. The peptide was extracted with 50% acetic acid (3 X 50 mL), the solvents evaporated *in vacuo*, and the product freeze-dried.

The crude peptide was initially purified on an Amberlite IRA-68 ion-exchange column; further purification was accomplished by RP-HPLC on a preparative C_{18} column. The solvents used were: water containing 0.1%

TFA (Buffer A) and CH_3CN - H_2O (4:1) containing 0.1% TFA (Buffer B). A linear gradient of 0-20% B over 100 minutes was used. The appropriate fractions containing the peptide were pooled, the solvents evaporated *in vacuo*, and the product freeze-dried (644 mg). The purified peptide gave satisfactory amino acid analysis.

AAA: Ala 18.42 (16), Arg 23.74 (24), Asx 10.41 (8), Gly 1.47 (1), Lys 25.91 (31), Pro 8.14 (8), Ser 23.68 (24), Thr 23.99 (24).

C. Synthesis of [Ser-Tyr-Gly-Arg-Lys-Lys-Arg-Arg-Gln-Arg-Arg-Arg-Ala-Pro-Gln-Gly-Ser-Gln]$_8$-Lys$_4$-Lys$_2$-Lys-Gly-OH: [(HIV-1 *TAT*$_{46-63}$) Multiple Antigenic Peptide] [SEQ ID NO: 24]

This peptide was synthesized as described above, using the appropriate protected amino acid derivatives. The purified peptide gave satisfactory amino acid analysis.

AAA: Ala 9.74 (8), Arg 46.5 (48), Glu 26.55 (24), Lys 23.21 (23), Pro 8.56 (8), Ser 10.95 (16), Tyr 7.52 (8). During the hydrolysis of the peptide, Ser undergoes decomposition resulting in low recoveries.

D. Other Multiple Antigenic Peptides

Making use of repeating fragments of the SIV *TAT* protein, the following multiple antigenic peptides can be produced using the techniques described above.

(Gln-Arg-Arg-Arg-Thr-Pro-Gln-Arg-Arg-Arg-Thr-Pro-Gln-Arg-Arg-Arg-Thr-Pro-Gln)$_8$-MAP [SEQ ID NO: 25].

(Pro-Lys-Lys-Thr-Lys-Thr-His-Pro-Lys-Lys-Thr-Lys-Thr-His-Pro-Lys-Lys-Thr-Lys-Thr-His)$_8$-MAP [SEQ ID NO: 26].

(Pro-Lys-Lys-Ala-Lys-Thr-His-Pro-Lys-Lys-Ala-Lys-Thr-His-Pro-Lys-Lys-Ala-Lys-Thr-His)$_8$-MAP [SEQ ID NO: 27].

Similarly, making use of repeating fragments of the HIV *TAT* protein, the following MAPs can be produced using methods analogous to those described above.

(Gln-Arg-Arg-Arg-Ala-Pro-Gln-Arg-Arg-Arg-Ala-Pro-Gln-Arg-Arg-Arg-Ala-Pro-Gln)$_8$-MAP [SEQ ID NO: 28].

(Tyr-Gly-Arg-Lys-Lys-Arg-Arg-Gln-Gly-Arg-Lys-Lys-Arg-Arg-Gln-Gly-Arg-Lys-Lys-Arg-Arg-Gln)$_8$-MAP [SEQ ID NO: 29].

EXAMPLE 3: IMMUNIZATION OF ANIMALS WITH ANTI-*TAT* MULTIPLE ANTIGENIC PEPTIDES

The following experiment is performed to evaluate whether immunization with a multiple antigenic peptide of this invention in alum adjuvant induces a high titer antibody response and whether the presence of such antibodies will prevent or ameliorate acute viremia and subsequent disease progression in rhesus monkeys inoculated with infectious SIV.

Young adult, male, colony born rhesus monkeys (*Maraca mulatta*) older than 2 years of age, but weighing less than 10 kg (a species commonly used in experimental models of SIV infection) are employed. The animals are fed a commercial primate diet, optionally supplemented with fresh fruit. Water is provided *ad libitum*. Animals are randomized into two treatment groups (peptide or placebo).

Prior to challenge infection with SIV, the animals are immunized intramuscularly (by thigh injection) on day 0, day 10 and monthly as necessary with either 200 micrograms of test peptide mixed with alum as an adjuvant

or a saline-adjuvant placebo, until suitable antibody titers are attained.

TABLE 1

Group	No.	Immunogen	Route	Peptide Dose	Vol.
1	10	placebo[1]	i.m.	0 μg	400 μL
2	2	test peptide	i.m.	200μg[2]	400 μL

[1] Saline adjuvant only (unimmunized control)
[2] Dissolved in saline and mixed 1:1 in an alum suspension

Serum for antibody testing is collected prior to initial immunization, on day 7 and on day 17 post immunization and then 7 days after each monthly injection until suitable antibody titers are attained. SIV antibody in serum is determined by both radioimmunoassay and ELISA and is confirmed by Western Blot analysis.

After suitable antibody titers are attained in the peptide immunized animals, all animals are challenged intravenously with 50 AID_{50}SIV delta B670 virus by way of the saphenous vein using a 23 gauge butterfly needle. Needles are flushed with PBS before and after virus to assure accurate delivery of material. The B670 SIV strain is a well-characterized viral isolate originally isolated at Tulane Regional Primate Research Center (TRPRC).

At selected times after infection the clinical, immunological and virological status of the animals are evaluated. For example, a lymph node biopsy is performed at 2 and 4 weeks after challenge, and a sample of blood is collected for the determination of antibody titers at week 4 after challenge and then monthly until death or completion of study. For all measurements of antibody,

the mean titer level, expressed on a $\log_{10}$ scale is computed at each time point.

Results of this protocol are anticipated to reveal significant protection of the animals by use of the peptide immunogens of this invention.

EXAMPLE 4: EVALUATION OF ANTIBODY TITERS OF IMMUNIZED MONKEYS WITH *TAT* IMMUNOGEN FOR SIV

Serum from monkeys immunized with the SIV $TAT_{80\text{-}95}$ multiple antigenic peptide are tested for the presence of anti-SIV TAT binding activity. This activity is assessed by two independent methods.

First [Tyr_0]-SIV $TAT_{80\text{-}95}$ is radiolabeled with ^{125}I by the pulse labeling method of Culler et al, Proc. Soc. Exp. Biol. Med., 173:264-268 (1985) and purified by partition chromatography [QUSO, Degussa Corporation, Dublin, Ohio] and subsequent high pressure liquid chromatography. The HPLC elution profile of [^{125}I-TYR_0]-SIV $TAT_{80\text{-}95}$ is illustrated in Fig. 1. Serial dilutions of the monkey serum are incubated with the [^{125}I-TYR_0]-SIV $TAT_{80\text{-}95}$ for 18-24 hours at room temperature. The antibodies present in the samples are precipitated, along with any bound [^{125}I-TYR_0]-SIV $TAT_{80\text{-}95}$, by subsequent incubation with goat anti-monkey serum followed by centrifugation. The supernatant is removed and the remaining pellet is counted in a gamma counter to determine the amount of [^{125}I-TYR_0]-SIV $TAT_{80\text{-}95}$ bound by the monkey serum dilutions. For comparison, titer is defined as the dilution of monkey serum required to bind 50% of the added [^{125}I-TYR_0]-SIV $TAT_{80\text{-}95}$ tracer.

Second, the monkey serum is titrated by enzyme-linked immunoassay (ELISA) on polystyrene microtiter plates that have previously been coated with both the SIV $TAT_{80\text{-}95}$ multiple antigenic peptide to determine the total binding activity, and with ovalbumin to determine amount

of the binding that is not specific for SIV TAT_{80-95}. Briefly, the monkey serum is serially diluted using 0.01M phosphate buffered saline containing 0.01% Tween 20 and 0.5% BSA (PBS/Tween 20). 100 µl of each serial dilution of monkey serum is added in triplicate to the wells of a 96-well microtiter plate coated with either SIV TAT_{80-95} multiple antigenic peptide or ovalbumin. The plates are incubated for 2 hours at 37°C with rocking. Following incubation, the plates are washed 5 times with the PBS/Tween 20 buffer. Goat anti-monkey IgG, labeled with horse-radish peroxidase (HRPO), is added to each well at the appropriate dilution in PBS/Tween 20 buffer. Following an incubation at 37°C for 1 hour, the plates are again washed 5 times. The specific HRPO substrate, Ultrablue (Transgenic Sciences, Inc., Milford, MA), is added and incubated in the dark for 30 minutes at room temperature. The reaction is terminated by adding 2.5N HCl to each well and the optical density of each well is measured at 450 nm. For comparison, the titer is defined as the concentration of monkey serum which results in an optical density reading of 1.0. The titers from each monkey are followed over time to determine empirically when the plateau antibody response against the SIV TAT_{80-95} multiple antigenic peptide is attained.

Numerous modifications and variations of the present invention are included in the above-identified specification and are expected to be obvious to one of skill in the art. Such modifications and alterations to the compositions and processes of the present invention are believed to be encompassed in the scope of the claims appended hereto.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Immunobiology Research, Institute Inc.

(ii) TITLE OF INVENTION: Vaccine Interdiction of Extracellular Transactivating Proteins of Human Immunodeficiency Virus and Other Chronically Infecting Viruses Employing Similar Intercellular Transactivating Strategies

(iii) NUMBER OF SEQUENCES: 38

(iv) CORRESPONDENCE ADDRESS:
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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
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(viii) ATTORNEY/AGENT INFORMATION:
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(C) REFERENCE/DOCKET NUMBER: IRI44PCT

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(A) TELEPHONE: 215-540-9200
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 261 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..258

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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ATG GAG CCA GTA GAT CCT AGA CTA GAG CCC TGG AAG CAT CCA   42
Met Glu Pro Val Asp Pro Arg Leu Glu Pro Trp Lys His Pro
  1               5                  10

GGA AGT CAG CCT AAA ACT GCT TGT ACC AAT TGC TAT TGT AAA   84
Gly Ser Gln Pro Lys Thr Ala Cys Thr Asn Cys Tyr Cys Lys
 15                  20                  25

AAG TGT TGC TTT CAT TGC CAA GTT TGT TTC ATA ACA AAA GCC  126
Lys Cys Cys Phe His Cys Gln Val Cys Phe Ile Thr Lys Ala
     30                  35                  40

TTA GGC ATC TCC TAT GGC AGG AAG AAG CGG AGA CAG CGA CGA  168
Leu Gly Ile Ser Tyr Gly Arg Lys Lys Arg Arg Gln Arg Arg
         45                  50                  55

AGA CCT CCT CAA GGC AGT CAG ACT CAT CAA GTT TCT CTA TCA  210
Arg Pro Pro Gln Gly Ser Gln Thr His Gln Val Ser Leu Ser
             60                  65                  70

AAG CAA CCC ACC TCC CAA TCC CGA GGG GAC CCG ACA GGC CCG  252
Lys Gln Pro Thr Ser Gln Ser Arg Gly Asp Pro Thr Gly Pro
                 75                  80

AAG GAA TAG                                             261
Lys Glu
 85
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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 86 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Met Glu Pro Val Asp Pro Arg Leu Glu Pro Trp Lys His Pro Gly
 1               5                  10                  15

Ser Gln Pro Lys Thr Ala Cys Thr Asn Cys Tyr Cys Lys Lys Cys
            20                  25                  30

Cys Phe His Cys Gln Val Cys Phe Ile Thr Lys Ala Leu Gly Ile
            35                  40                  45

Ser Tyr Gly Arg Lys Lys Arg Arg Gln Arg Arg Arg Pro Pro Gln
            50                  55                  60

Gly Ser Gln Thr His Gln Val Ser Leu Ser Lys Gln Pro Thr Ser
            65                  70                  75

Gln Ser Arg Gly Asp Pro Thr Gly Pro Lys Glu
            80                  85
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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
Ser Tyr Gly Arg Lys Lys Arg Arg Gln Arg Arg Arg Ala Pro
 1               5                  10

Gln Gly Ser Gln
 15
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Ser Tyr Gly Arg Lys Lys Arg Arg Gln Arg Arg Arg Ala Pro
1 5 10

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Ser Tyr Gly Lys Lys Lys Arg Arg Gln Arg Arg Arg Ala Pro
1 5 10

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Ser Tyr Gly Arg Lys Lys Arg Lys Gln Arg Arg Arg Ala Pro
1 5 10

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Ser Tyr Gly Arg Lys Lys Arg Arg Pro Arg Arg Arg Ala Pro
1 5 10

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Ser Tyr Gly Arg Lys Lys Arg Arg Gln Arg Gln Arg Ala Pro
1 5 10

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Ser Tyr Gly Arg Lys Lys Arg Arg Gln Arg Arg Gly Ala Pro
1 5 10

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Ser Tyr Gly Arg Lys Lys Arg Arg Gln Arg Arg Arg Thr Pro
1 5 10

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Ser Tyr Gly Arg Lys Lys Arg Arg Gln Arg Arg Arg Pro Pro
1 5 10

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Ser Tyr Gly Arg Lys Lys Arg Arg Gln Arg Arg Arg Ala His
1 5 10

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Arg Arg Arg Thr Pro Lys Lys Thr Lys Ala Asn Thr Ser Ser
1 5 10

Ala Ser Tyr
15

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Tyr Glu Gln Gln Arg Arg Arg Thr Pro Lys Lys Thr Lys Ala
1 5 10

Asn Thr Ser Ser Ala Ser
15 20

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Cys Phe Ile Thr Lys Ala Leu Gly Ile Ser Tyr Gly Arg Lys
1 5 10

Lys Arg Arg Gln Arg Arg Arg Pro Pro Gln Gly Ser Gly Thr
15 20 25

His Gln Val Ser Leu Ser Lys Gln
30 35

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Gln Arg Arg Arg Ala Pro
1 5

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Gly Arg Lys Lys Arg Arg Gln
1 5

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Pro Lys Lys Thr Lys
1 5

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Pro Lys Lys Ala Lys
1 5

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Gln Arg Arg Arg Thr Pro
1 5

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Arg Arg Arg Ala Pro
1 5

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Tyr Arg Arg Arg Thr Pro Lys Lys Thr Lys Ala Asn Thr Ser
1 5 10

Ser Ala Ser
15

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Arg Arg Arg Thr Pro Lys Lys Thr Lys Ala Asn Thr Ser Ser
1 5 10

Ala Ser Lys Lys Lys Lys Lys Lys Lys Gly
15 20

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Ser Tyr Gly Arg Lys Lys Arg Arg Gln Arg Arg Arg Ala Pro
1 5 10

Gln Gly Ser Gln Lys Lys Lys Lys Lys Lys Gly
15 20 25

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Gln Arg Arg Arg Thr Pro Gln Arg Arg Arg Thr Pro Gln Arg
1 5 10

Arg Arg Thr Pro Gln
15

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Pro Lys Lys Thr Lys Thr His Pro Lys Lys Thr Lys Thr His
1 5 10

Pro Lys Lys Thr Lys Thr His
15 20

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Pro Lys Lys Ala Lys Thr His Pro Lys Lys Ala Lys Thr His
1 5 10

Pro Lys Lys Ala Lys Thr His
15 20

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Gln Arg Arg Arg Ala Pro Gln Arg Arg Arg Ala Pro Gln Arg
1 5 10

Arg Arg Ala Pro Gln
15

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Tyr Gly Arg Lys Lys Arg Arg Gln Gly Arg Lys Lys Arg Arg
1 5 10

Gln Gly Arg Lys Lys Arg Arg Gln
15 20

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Arg Arg Arg Ala Pro
1 5

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Arg Gln Arg Ala Pro
1 5

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Arg Arg Gly Ala Pro
1 5

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Arg Arg Arg Thr Pro
1 5

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

Arg Arg Arg Pro Pro
1 5

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

Arg Arg Arg Ala His
1 5

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

Arg Lys Lys Arg Arg
1 5

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

Lys Lys Lys Arg Arg
1 5

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

Arg Lys Lys Arg Lys
1 5

WHAT IS CLAIMED IS:

1. An isolated immunogen comprising an amino acid sequence comprising the cellular uptake region or a fragment thereof of a native transactivating (*TAT*) protein from a selected virus, said immunogen capable of eliciting antibody against said native protein.

2. The immunogen according to claim 1 wherein said protein is a *TAT* protein of human immunodeficiency virus (HIV) or simian immunodeficiency virus (SIV).

3. The immunogen according to claim 2 wherein said sequence comprises a *TAT* protein having amino acid deletions at the amino terminus.

4. The immunogen according to claim 2 wherein said sequence comprises a *TAT* protein sequence having amino acid deletions or substitutions at one or more cysteine residues which normally participate in disulfide bonding.

5. The immunogen according to claim 3 wherein said deletion comprises about 3 to about 10 amino acids at the amino terminus.

6. The immunogen according to claim 1 wherein said cellular uptake region is an HIV *TAT* sequence selected from the group consisting of

SYGRKKRRQRRRAPQGSQ	SEQ ID NO: 3,
SYGRKKRRQRRRAP	SEQ ID NO: 4,
SYGKKKRRQRRPAP	SEQ ID NO: 5,
SYGRKKRKQRRRAP	SEQ ID NO: 6,
SYGRKKRRPRRRAP	SEQ ID NO: 7,
SYGRKKRRQRQRAP	SEQ ID NO: 8,
SYGRKKRRQRRGAP	SEQ ID NO: 9,

SYGRKKRRQRRRTP SEQ ID NO: 10,
SYGRKKRRQRRRPP SEQ ID NO: 11,
SYGRKKRRQRRRAH, and SEQ ID NO: 12,
fragments and allelic variants thereof.

7. The immunogen according to claim 6 wherein said fragment is selected from the group consisting of RRRAP, RQRAP, RRGAP, RRRTP, RRRPP, RRRAH, RKKRR, KKKRR, and RKKRK (SEQ ID NOS: 30-38).

8. The immunogen according to claim 1 wherein said cellular uptake region is an SIV *TAT* sequence selected from the group consisting of RRRTPKKTKANTSSASY (SEQ ID NO: 13), YEQQRRRTPKKTKANTSSAS (SEQ ID NO: 14), and fragments and allelic variants thereof.

9. The immunogen according to claim 8 wherein said fragment is selected from the group consisting of PKKAK (SEQ ID NO: 19), PKKTK (SEQ ID NO: 18) and RRRTP (SEQ ID NO: 33).

10. The immunogen according to claim 1 comprising a synthetic peptide from a selected region of said protein coupled to a carrier.

11. The immunogen according to claim 10 comprising a construct comprising a plurality of synthetic peptides from said protein in the form of a multiple antigenic peptide.

12. The immunogen according to claim 1 wherein said sequence does not share the biological activity of the native protein.

13. A composition useful for inducing an immune response in a patient protective against infection with a virus characterized by a transactivating protein, the composition comprising an immunogen capable of eliciting antibody against a native transactivating protein from said virus, said immunogen comprising an amino acid sequence comprising the cellular uptake region of said protein or a fragment thereof in a selected pharmaceutical carrier.

14. The composition according to claim 13 wherein said carrier comprises an adjuvant selected from the group consisting of alum, liposomes, and magnesium hydroxide.

15. The composition according to claim 13 comprising an immunogen of any of claims 2-12.

16. The composition according to claim 13 comprising at least two different immunogens.

17. An isolated nucleic acid sequence encoding the immunogen of any of claims 1 to 12.

18. The sequence according to claim 17, said sequence suitable for direct administration into a mammal and *in vivo* expression of said immunogen.

19. A nucleic acid molecule comprising the sequence of claim 17 in association with nucleic acid sequences capable of regulating the replication and expression of said *TAT* sequence *in vivo* in said mammal or *in vitro* in a host cell culture.

20. An antibody capable of identifying a virus strain having a selected extracellular *TAT* protein, wherein said antibody is capable of binding to an immunogen of any of claims 1-12.

21. A method of producing an antibody capable of identifying a virus strain having an extracellular *TAT* protein, said method comprising immunizing an animal with an immunogen of any of claims 1-12 and isolating said antibody from the tissue of said animal.

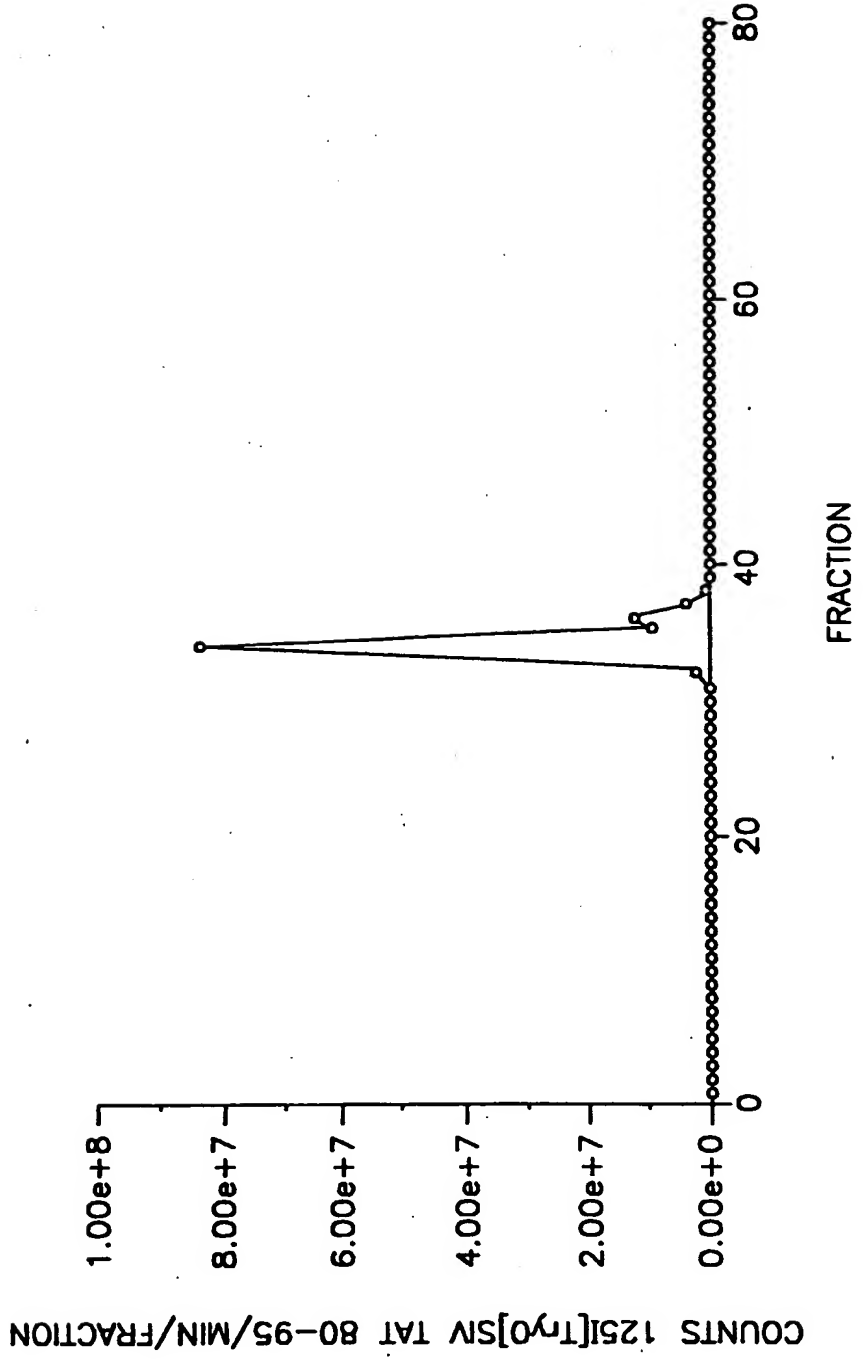
22. The use of an immunogen of any of claim 1 to 12 or a nucleic acid sequence of claim 17 in preparing a medicament for immunizing a patient against infection with a virus characterized by a transactivating protein.

23. The use of an immunogen of any of claim 1 to 12 or a nucleic acid sequence of claim 17 in preparing a medicament for reducing viremia in a mammal infected with a virus characterized by a transactivating protein.

FIGURE 1

```
ATG GAG CCA GTA GAT CCT AGA CTA GAG CCC TGG AAG CAT    39
Met Glu Pro Val Asp Pro Arg Leu Glu Pro Trp Lys His
  1               5                  10

CCA GGA AGT CAG CCT AAA ACT GCT TGT ACC AAT TGC TAT    78
Pro Gly Ser Gln Pro Lys Thr Ala Cys Thr Asn Cys Tyr
     15                  20                  25

TGT AAA AAG TGT TGC TTT CAT TGC CAA GTT TGT TTC ATA   117
Cys Lys Lys Cys Cys Phe His Cys Gln Val Cys Phe Ile
             30                  35

ACA AAA GCC TTA GGC ATC TCC TAT GGC AGG AAG AAG CGG   156
Thr Lys Ala Leu Gly Ile Ser Tyr Gly Arg Lys Lys Arg
 40                  45                  50

AGA CAG CGA CGA AGA CCT CCT CAA GGC AGT CAG ACT CAT   195
Arg Gln Arg Arg Arg Pro Pro Gln Gly Ser Gln Thr His
         55                  60                  65

CAA GTT TCT CTA TCA AAG CAA CCC ACC TCC CAA TCC CGA   234
Gln Val Ser Leu Ser Lys Gln Pro Thr Ser Gln Ser Arg
                 70                  75

GGG GAC CCG ACA GGC CCG AAG GAA TAG                  261
Gly Asp Pro Thr Gly Pro Lys Glu
     80                  85
```

FIG. 2


COUNTS 125I[Tyr0]SIV TAT 80-95/MIN/FRACTION
1.00e+8
8.00e+7
6.00e+7
4.00e+7
2.00e+7
0.00e+0
0
20
40
60
80
FRACTION

INTERNATIONAL SEARCH REPORT

Int. ..ational application No.
PCT/US95/06077

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A61K 39/116; C07K 5/00, 7/00; C07H 19/00, 19/22

US CL : 424/204.1; 530/326; 536/27.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/204.1; 530/326; 536/27.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, Dialog, search terms: tat protein, HIV, synthetic peptides, multiple antigen peptide, gene therapy, vaccines, immunogen.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Proceedings of the National Academy of Sciences, Volume 83, issued September 1986, A. Aldovini et al, "Synthesis of the Complete Trans-activation gene product of Human T-lymphotrophic Virus Type III in Escherichia coli:Demonstration of Immunogenicity in vivo and expression in vitro", pages 6672-6676, see entire article.	1-7, 9-11, 13, 15, 16
Y	Proceedings of the National Academy of Sciences, Volume 86, issued October 1989, A. D. Frankel et al, "Activity of Synthetic Peptides From the Tat Protein of Human Immunodeficiency Virus Type 1", pages 7397-7401, see entire article.	1-14, 16

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 09 AUGUST 1995	Date of mailing of the international search report 25SEP 1995
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer LYNETTE F. SMITH Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

In. ..ational application No.
PCT/US95/06077

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Proceedings of the National Academy of Sciences, Volume 85, issued August 1988, J. P. Tam, "Synthetic Peptide Vaccine Design:Synthesis and Properties of a High-Density Multiple Antigenic Peptide System", pages 5409-5413, see entire article.	11
Y	Proceedings of the National Academy of Sciences, Volume 91, issued January 1994, S. Fawell et al, "Tat-mediated Delivery of Heterologous Proteins Into Cells", pages 664-668, especially pages 664-665.	1-12

INTERNATIONAL SEARCH REPORT

Int...ational application No.
PCT/US95/06077

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 15, 17-23
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.